Chemosphere 74 (2009) 890–895

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Interactions of mixed organic contaminants in uptake by rice seedlings

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ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 14 October 2008
Accepted 30 October 2008
Available online 17 December 2008

Keywords:
Plant uptake
Hydroponic solution
Organic contaminants
Rice seedlings
Transpiration rate

ABSTRACT

Uptakes of *o*-chlorophenol (CP), 2,4-dichlorophenol (DCP), trichloroethylene (TCE), and atrazine (ATR), as single and mixed contaminants, by roots and shoots of rice seedlings (*Oryza sativa* L.) from hydroponic solutions were measured following a 48-h exposure of plant roots. As single contaminants, the concentrations of CP, DCP, and ATR in rice roots and shoots increased significantly with increasing concentrations in external solutions; however, TCE concentrations in rice roots and shoots decreased with increasing external TCE concentration or the exposure time. The observed bioconcentration factors (BCFs) of CP and DCP with roots and the BCF of ATR with shoots approximated the equilibrium values according to the partition-limited model. The BCF of DCP with shoots was about 30% of the partition limit, due to insufficient water transport into plants for DCP. In the ATR–CP–DCP mixed system, the BCFs of CP and DCP with both roots and shoots decreased significantly with increasing contaminant concentrations due to the enhanced mixed-contaminant phytotoxicity, as manifested by the greatly reduced plant transpiration rate. In the ATR–CP–DCP mixture system, the BCFs of ATR with roots at low concentrations were comparable with those for ATR alone, whereas the BCFs increased at high concentrations for an unknown reason. In the TCE-DCP system, TCE concentrations in roots increased with increasing TCE in external solutions, while TCE concentration in shoots stayed steady because of the strong TCE exchange at the air-leaf interface. The BCF of DCP with roots was comparable with that of DCP alone because there was no significant effect of added TCE on the plant transpiration rate.

1. Introduction

Food crops are susceptible to contamination by various pesticides and organic wastes when exposed to a polluted source, as these chemicals may be transported to varying extents into crop tissues (Harris and Sans, 1967; Walker, 1972; Li et al., 2002; Wild et al., 2006). The need for better understanding the mechanism and influential factors on plant uptake has prompted a series of studies on the plant-uptake process in recent times (Riederer, 1990; Paterson et al., 1994; Trapp, 1995; Trapp and Mathies, 1995; Burken and Schnoor, 1997; Weiss, 2000; Li et al., 2002, 2005; Wild et al., 2005). Analyses of the concentrations of nonionic contaminants in plants in relation to the external levels in water (or soil solution) from extensive sources have revealed that these contaminants enter plants largely via a passive (i.e., partition) process (Briggs et al., 1982; Chiou et al., 2001; Trapp, 2004; Su and Zhu, 2006). The magnitude and efficiency of plant uptake depends in principle on contaminant level and properties, plant species/composition, exposure time, and other variables (Briggs et al., 1982; Chiou et al., 2001; Trapp, 2004; Wild et al., 2005). Previously, we have studied the uptake of atrazine by rice seedlings from nutrient solution with and without coexisting organic and metal-ion species (Su et al., 2005; Su and Zhu, 2006). It was found that the uptake of atrazine (ATR) by rice seedlings was largely unaffected by co-existing organic compounds and appeared to proceed essentially by passive (i.e., partition) mechanism. In current literature, there have been few studies comparing contaminant levels in different plant parts and most published studies have been conducted in single- rather than mixed-contaminant systems. In view of the occurrence of various organic contaminants in natural systems, more extensive tests are necessary to understand the interactions of organic contaminants on their concurrent uptakes by plants.

ATR is one of the most intensively used herbicides in agricultural practices (e.g., maize production). Residues of ATR spread across the land surface and soil-water interface to the groundwater region (Belluck et al., 1991; Burkart and Kolpin, 1993). Previous studies indicated that ATR can greatly reduce the transpiration rate of rice seedlings at low concentrations (Su and Zhu, 2006). ATR applied to rotation fields significantly affects the growth of rice in

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doi:10.1016/j.chemosphere.2008.10.057

China, as the rice seedlings accumulate significant levels of ATR from soil solution. On the other hand, trichloroethylene (TCE) has been found in groundwater and many surface waters as a result of the manufacturing, use, and disposal of the chemical; phenolic compounds, such as o-chlorophenol (CP) and 2,4-dichlorophenol (DCP), may enter surface water upon the discharge of industrial wastewaters. As such, mixtures of these organic compounds may occur in agricultural fields.

In the present study, the uptakes of ATR, CP, TCE, and DCP from nutrient solutions as single and mixed contaminants by seedlings of a rice species have been determined for better understanding the interaction of co-existing contaminants on the plant uptake process. The concentrations for each parent contaminant in plant roots and shoots were quantified separately and analyzed in terms of plant transpiration rates and contaminant partition coefficients with individual cut-dried roots and shoots. This novel approach provides a unique basis for validating the plant-uptake data. Rice plant is chosen for investigation because it is a staple food crop in China that is susceptible to contamination by various pesticides and other chemicals from local pollution sources. Furthermore, there are few published studies on rice-plant uptake of anthropogenic organic substances in the literature. While ATR (a widely used herbicide and a weak base), chlorophenols (common wastewater contaminants and weak acids) and TCE (a volatile organic compound) represent different chemical types, they also exhibit significantly different partition coefficients to aid in the evaluation of the plant-uptake process.

2. Materials and methods

2.1. Preparation of rice seedlings

Seeds of *Oryza sativa* L. Chinese rice cv. Jiahua-1 were disinfected in 30% H_2O_2 (wt:wt) solution for 10 min, followed by a thorough washing with deionized water. The seeds were germinated in moist perlite in an incubator at 25 °C. Seven days after germination, the seedlings were transferred to a nutrient solution for continuing growth. Three weeks later, uniform seedlings were selected and transplanted to polyvinylchloride (PVC) pots (7.5 cm in diameter and 14 cm in height) for uptake experiments with a density of one plant per pot; each pot containing 500 ml nutrient solution with selected contaminants. The composition of the nutrient solution for the experiment was as follows: 5 mM NH_4NO_3, 12 mM KH_2PO_4, 2 mM K_2SO_4, 4 mM $CaCl_2$, 1.5 mM $MgSO_4$, 100 μM Fe(III)-ethylenediaminetetraacetic acid (EDTA), 10 μM H_3BO_4, 1.0 μM $ZnSO_4$, 1.0 μM $CuSO_4$, 5.0 μM $MnSO_4$, 0.5 μM Na_2MoO_4, 0.2 μM $CoSO_4$. The nutrient solution was changed once a week, and its pH value was adjusted to 5.5 using 0.1 M KOH or HCl solution.

2.2. Treatments with contaminants

Compounds used for plant-uptake studies, *o*-chlorophenol (CP), 2,4-dichlorophenol (DCP) and trichloroethylene (TCE) were all of analytical grade, and atrazine (ATR), were supplied by Dima Chemical Company, China (purity > 97%), and used as received without further treatment.

In plant-uptake experiments, a series of three-seedling sets (grown three weeks in PVC pots) of comparable sizes were prepared, with their roots being immersed into same nutrient solutions (ca. 500 mL each). The nutrient solutions containing different added levels of given contaminants were held by a series of identical PVC cylinders. The cylinder was fitted with a PVC septum (7.5 cm in diameter and 0.5 cm in thickness) with a hole drilled at its center (1.5 cm in diameter), through which the plant shoots extended into the external air. The section of the shoots passing through the septum hole was wrapped with sponge sheets to minimize the open space. This design prevented direct water evaporation from the nutrient solution into external air when the seedlings were in place. Hence, the mass of water transpired through plant shoots could be determined accurately by measuring the net weight change of the nutrient solution and plants.

In uptake studies of single contaminants, the initial contaminant concentrations in nutrient solutions were set at 10, 20, 40, 80, and 160 mg L^{-1} for CP; 5,10, 20, 40, and 80 mg L^{-1} for DCP; 2, 4, 8, and 16 mg L^{-1} for TCE; and 2, 4, 6, 8, and 10 mg L^{-1} for ATR. In uptake studies with CP, DCP, and ATR as mixed contaminants, the initial CP/DCP/ATR concentrations were set at 10/5/2, 20/10/4, 40/20/6, 80/40/8, and 160/80/10 mg L^{-1}. In uptake studies with DCP and TCE as mixed contaminants, the initial DCP/TCE concentrations were set at 0.5/2, 1/4, 2/8, and 4/16 mg L^{-1}.

Experiments were carried out for 48 h in an environmentally-controlled growth chamber that maintained a daily 14-h light period (260–350 μmol m^{-2} s^{-1}) and 25 °C for daytime and 20 °C for nighttime. The relative humidity was set at 70%.

2.3. Water transpiration rate

The amounts of water transpired by plants over the 48-h-period were determined based on the cumulated weight losses of the plant-solution systems by measuring their weight changes at 1–3 h intervals between 7:00 am and 11:00 pm. After each measurement, the amount of water lost by plant transpiration was replenished with fresh nutrient solution to maintain the total solution volume nearly constant at ca. 500 mL. By this method, the error in amounts of water transpiration was minimized. It also enabled a quick detection of the change in plant transpiration rate when the plant growth was strongly affected by the contaminant toxicity. From the cumulated water-loss weights and the measured fresh plant weights after 48-h exposure, the plant transpiration rates (G) (as g water/g fresh plant weight) were then calculated.

2.4. Plant-contamination levels

After harvest, the studied plants were removed from the nutrient solutions, rinsed with distilled water, blotted dry with tissue paper, and sectioned into roots and shoots, and the relative weight fractions of fresh roots and shoots (FW) were determined. The samples were ground with a mortar and pestle and subsequently extracted and analyzed for contaminant levels. The detailed analytical procedures are presented later. Control seedlings (grown in solutions without contaminants over the same period) were then removed and sectioned into roots and shoots; the relative weight fractions of fresh roots and shoots (FW) were determined. Halves of the sectioned roots and shoots of the control plants were dried in an oven (60–70 °C) for 24 h to determine their respective water contents.

2.5. Sorption with cut-dried roots/shoots

The combined cut-dried roots or shoots of control plants were ground into small particle sizes (passing 100 mesh), which were subsequently used to determine the K_{pom}, the contaminant partition coefficient between plant organic matter and water. To determine the K_{pom}, fixed amounts of dried ground roots or shoots (0.2 g) were added to a series of nutrient solutions (10 mL) containing different initial levels of a given contaminant; the suspensions were equilibrated for 48 h and contaminant concentrations in roots (or shoots) and water were determined after this equilibration period. Average values from duplicate samples were used to construct the sorption isotherms. This equilibration period was